(2)

FINAL TECHNICAL REPORT FOR OFFICE OF NAVAL RESEARCH CONTRACT N00014-85-K-0061 FROM 1 DECEMBER 1985 TO 30 NOVEMBER 1989

PRINCIPAL INVESTIGATOR: RALPH MITCHELL
DIVISION OF APPLIED SCIENCES, HARVARD UNIVERSITY
CAMBRIDGE, MASSACHUSETTS 02138

1990

SUMMARY OF WORK ACCOMPLISHED

This contract supported basic research in two separate but related projects. The first project (I) involved the study of interactions between microorganisms, primarily bacteria, and marine invertebrates and macroalgae. This research focused on the role of bacterial films in the settlement and metamorphosis of marine invertebrate larvae. The second project (II) involved the study of interactions between marine bacteria and various substrata during the process of bacterial adhesion. During the period of this contract a total of twelve separate manuscripts were published: nine in refereed journals, two chapters in books, and one in the proceedings of a conference. These publications are listed at the end of this report.

I. In the course of the first project, we examined the role of primary microbial films in subsequent macrofouling. In this research, the attachment of larvae from two fouling sessile invertebrates, the balanomorph barnacle, Balanus amphitrite, and a bryozoan, Bugula neritina, as well as zoospores from a green macroalga, Enteromorpha sp., was examined on filmed and unfilmed substrata. Reports we published showed the following:

(1) that excess concentrations of some ions inhibited barnacle settlement while dibuteryl cAMP was stimulatory (Rittschof, Maki, Mitchell & Costlow 1986);

(2) films composed of individual species of bacteria could inhibit the attachment of barnacle larvae (Maki, Rittschof, Costlow & Mitchell 1988; Mitchell & Maki 1988) and bryozoan larvae (Mitchell & Maki 1988; Maki, Rittschof, Schmidt, Snyder & Mitchell 1989);

(3) a single species of bacteria may possess a number of biochemical cues or other factors, some of which are stimulatory to certain larvae but inhibitory to others (Mitchell & Maki 1988);

(4) factors in addition to substratum surface energy determine attachment of bryozoan larvae especially when bacterial films are present (Maki, Rittschof, Schmidt, Snyder & Mitchell 1989);

(5) increases in surface energy caused by the presence of microbial films may contribute to increased adhesion by algal swarmers (Dillon, Maki & Mitchell 1989);

(6) films of the same bacterium adsorbed on different substrata elicit different attachment responses by the larvae (Maki, Rittschof, Samuelsson, Szewzyk, Yule, Kjelleberg, Costlow & Mitchell 1990);

(7) the exopolymers of the bacteria appear to be involved in the larval attachment response (Maki, Rittschof, Samuelsson, Szewzyk, Yule, Kjelleberg, Costlow & Mitchell 1990).

Additional research supported in part by this contract showed that:

(a) gut bacteria from the flounder and squid undergo a starvation response similar to that reported for planktonic bacteria (i.e., fragmentation, dwarfing, change in cell-surface characteristics) when their animal hosts are starved (Conway, Maki, Mitchell & Kjelleberg 1986);

(b) methylotrophs, bacteria capable of using reduced C-1 compounds as their carbon and energy sources, may occur as intracellular symbionts in mussels found near hypersaline seeps (Cavanaugh, Levering, Maki, Mitchell & Lidstrom 1987);

(c) mollicutes can exist in non-disease associations with aquatic invertebrate larvae (Boyle, Maki & Mitchell 1987).

In addition to this work two reviews were completed, the first on the role of lectins in interactions between aquatic organisms (Maki & Mitchell 1986), the second on bacterial adhesion and its consequences (Maki & Mitchell 1989).

II. The results obtained during the course of the second project on substratum-microorganism interactions and the adhesion of marine bacteria are summarized as follows:

(1) a series of field experiments indicated that there are substratum influences on the attachment of bacteria and that the processes controlling bacterial adhesion to substrata in antarctic waters occur within 15 min after the surfaces are placed in the water (Maki, Little, Wagner & Mitchell 1990).

(2) Previously our data indicated that barnacles attached in significantly different numbers on films of a single bacterium when the films were adsorbed to different substrata. When transmission electron microscopy was used to examine the adhesion of these bacterial cells to the different surfaces, the cells were all observed to have the same orientation in relation to the surface on each substratum. Results were not due to the preferential adhesion of certain portions of the cell to the substratum. This suggests that adhesion of the same bacterium to different substrata must be controlled by the chemical specificity of the bacterial exopolymer. Attachment responses of the barnacle larvae may be due to their ability to detect surface changes on the attached cells.

(3) Enteric bacteria were conjugated with marine bacteria to insert transposons into the latter. These insertions caused mutations that changed the exopolymers of the bacteria.

These changes in exopolymer affected the ability of the cells to irreversibly attach to a number of different substrata.

(4) Starvation of a copiotrophic marine bacteria resulted in the response of fragmentation (i.e., cell division without growth) forming dwarf cells. Vesicles were also observed using transmission electron microscopy on the surface of the starved cells. Protein synthesis in the starved cells could be blocked with chloramphenicol suggesting that antibiotics and/or antimetabolites could be used to examine the role of certain metabolic processes in the adhesion of starved bacteria.

Further work on sections II. 2, 3 and 4 is continuing on a separate grant from ONR.

PUBLICATIONS INVOLVING THIS CONTRACT 1985-1989

1. Maki JS, Mitchell R. 1986. The function of lectins in interactions among marine bacteria, invertebrates, and algae. Pages 409-425, in Mirelman D (ed), Microbial Lectins and Agglutinins: Properties and Biological Activity. John Wiley & Sons, New York

2. Conway PL, Maki J, Mitchell R, Kjelleberg S. 1986. *Starvation of marine flounder,* squid and laboratory mice and its effect on the intestinal microbiota. FEMS Microbiology Ecology 38: 187-195

3. Rittschof D, Maki J, Mitchell R, Costlow JD. 1986. Ion and neuropharmacological studies of barnacle settlement. Netherlands Journal of Sea Research 20: 269-275

4. Cavanaugh CM, Levering PR, Maki JS, Mitchell R, Lidstrom ME. 1987. Symbiosis of methylotrophic bacteria and deep-sea mussels. Nature 325: 346-348

5. Boyle PJ, Maki JS, Mitchell R. 1987. Mollicute identified in novel association with aquatic invertebrate. Current Microbiology 15: 85-89

6. Maki JS, Rittschof D, Costlow JD, Mitchell R. 1988. Inhibition of attachment of larval barnacles, Balanus amphitrite, by bacterial surface films. Marine Biology 97: 199-206

7. Mitchell R, Maki JS. 1988. Microbial surface films and their influence on larval settlement and metamorphosis in the marine environment. Pages 489-497 in, Thompson M-F, Sarojini R, Nagabhushanam R (eds), Marine Biodeterioration: Advanced Techniques Applicable to the Indian Ocean. Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi

8. Dillon PS, Maki JS, Mitchell R. 1989. Adhesion of Enteromorpha swarmers to microbial films. Microbial Ecology 17: 39-47

9. Maki JS, Mitchell R. 1989. L'adhesion microbienne aux surfaces et ses consequences. Pages 387-409 in, Bianchi M, Marty D, Bertrand J-C, Caumette P, Gauthier M (eds), Micro-organismes dans les Ecosystemes Oceaniques. Masson, Paris

10. Maki JS, Rittschof D, Schmidt AR, Snyder AG, Mitchell R. 1989. Factors controlling attachment of bryozoan larvae: a comparison of bacterial films and unfilmed surfaces. Biological Bulletin 177: 295-302

11. Maki JS, Little BJ, Wagner P, Mitchell R. 1990. Biofilm formation on metal surfaces in Antarctic waters. Biofouling 2: 27-38

12. Maki JS, Rittschof D, Samuelsson M-O, Szewzyk U, Yule AB, Kjelleberg S, Costlow JD, Mitchell R. 1990. Effect of marine bacteria and their exopolymers on the attachment of barnacle cypris larvae. Bulletin of Marine Science 46: 499-511